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Plasma B-type natriuretic peptide study in children with severe enterovirus 71 infection: a pilot study

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SUMMARY

Objectives: Severe enterovirus 71 (EV71) infections in children can result in acute heart failure. B-type natriuretic peptide (BNP) is a good biomarker of myocardial stress. The purpose of this study was to use plasma BNP for the detection of EV71 infection with cardiac involvement.
Methods: Patients with severe EV71 infections and healthy control subjects were studied: group 1 ($n = 30$), normal controls; group 2 ($n = 20$), EV71 infection with central nervous system involvement; and group 3 ($n = 3$), EV71 infection with cardiopulmonary failure. The demographic and laboratory data including plasma BNP levels were analyzed statistically.
Results: All group 2 patients recovered completely without neurological sequelae, and all group 3 patients survived without cardiac complications. Group 3 patients had higher troponin I, MB fraction of creatine kinase, and BNP levels than patients of the other groups. The median BNP values were <5 pg/ml in group 1, 9.5 pg/ml in group 2, and 238 pg/ml in group 3. Using a BNP cut-off value of 100 pg/ml to identify cases with severe EV71 infection and acute heart failure, the sensitivity and specificity were both 100%.
Conclusions: Children with severe EV71 infections have varying degrees of myocardial stress. Plasma BNP would be a sensitive and reliable biomarker for the detection of cardiac involvement in children with severe EV71 infections.

1. Introduction

Severe enterovirus 71 (EV71) infections are unusual during an epidemic of EV71, but they may result in high morbidity and mortality.[1–3] Most children with EV71 infections present with hand, foot, and mouth disease (HFMD) or herpangina, and less than 0.01% of children with EV71 infections develop more serious symptoms such as central nervous system (CNS) manifestations, pulmonary edema, acute heart failure, rapid shock, or even death.[4–6] Acute cardiac dyskinesia, low ejection fraction (EF), and regional wall motion abnormalities of the left ventricle detected by echocardiography have commonly been noted in EV71-related early mortality.[6–8] Therefore, acute heart failure with or without pulmonary edema has been proposed as the main cause of rapid deterioration leading to EV71-related early mortality.

B-type natriuretic peptide (BNP), which is also called brain-type natriuretic peptide, was first described in 1988 after its isolation from porcine brain.[9] However, it was soon found to originate mainly from the heart, representing a cardiac hormone secreted predominantly from the ventricular myocardium.[10] The main stimulus for increased BNP synthesis and secretion is myocardial wall stress caused by excessive volume or pressure loading of the heart.[11,12] In clinical application, BNP has been thought to be a sensitive and specific biomarker of ventricular dysfunction and heart failure. Correlations between elevated plasma BNP concentrations in patients with heart failure and the severity of heart failure symptoms have been found to predict hospital readmission and mortality, and increased during different types of myocardial overload.[11–20].

There is now increasing interest in BNP, not only in adults with heart failure, but also in pediatric patients with cardiovascular diseases such as congenital heart disease, critical heart disease, and Kawasaki disease.[13–20] In the pediatric age group, BNP can be measured on commercial laboratory platforms and is now widely used. However, the clinical application of plasma BNP remains

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limited for children who have severe EV71 infections with or without acute heart failure.

The purpose of this pilot study was to determine plasma BNP levels in the different stages of severe EV71 infection and to establish a method of clinically applying plasma BNP in the treatment of children with severe EV71 infection.

2. Materials and methods

2.1. Study population

Children younger than 7 years old with severe EV71 infections who were admitted to the pediatric intensive care unit (PICU) of Taichung Veterans General Hospital between April and July 2012 were enrolled in this study. Severe EV71 infections diagnosed clinically were laboratory-confirmed by the Taiwan Center for Disease Control. The methods used to identify EV71 were the same as those described by Fu et al.[8] Thirty healthy children younger than 7 years old constituted the control group. They visited the outpatient clinics for heart murmur, chest pain, or allergic rhinitis. A detailed history and complete physical examination were conducted for each case. Functional murmur and non-cardiac chest pain were confirmed by clinical features and laboratory studies, such as transthoracic echocardiography, chest X-ray, and complete electrocardiogram. Children with allergic rhinitis received echocardiography and a complete electrocardiogram to exclude any cardiac problems. Written informed consent was obtained from a parent for all children included in this study. Patients who had genetic disorders, neuromuscular diseases, craniofacial abnormalities, or previous cardiovascular diseases, and those who could not participate for other reasons, were excluded. The study cohort was divided into three groups: group 1 comprised normal controls, group 2 included patients who had stage 2 EV71 infections, and group 3 included patients who had stage 3 EV71 infections. In this study, two patients who were suspected to have a stage 2 EV71 infection initially were excluded due to negative results for EV71 in the laboratory confirmation study.

2.2. Staging of children with EV71 infection

According to a previous study on the staging of EV71 infection,[3] the clinical features of EV71 infection can be divided into three stages: HFMD/herpangina (stage 1) with oro-mucocutaneous lesions only; CNS involvement (stage 2) with subtle neurologic symptoms or signs such as drowsiness, limb weakness, ataxia, and myoclonic jerks; and cardiopulmonary failure (stage 3) associated with pulmonary edema/hemorrhage or acute decreased left ventricular systolic function and shock. Severe EV71 infection was defined as an EV71 infection at or beyond stage 2.

2.3. Echocardiography

Echocardiography studies were performed using standard techniques in accordance with the recommendations of the Committee on M-mode Standardization of the American Society of Echocardiography.[21] All of the subjects received a complete echocardiogram including M-mode, two-dimensional (2D) Doppler, and color Doppler echocardiograms. Left ventricular systolic function was evaluated by EF and FS from M-mode and/or Simpson's rule by 2D echocardiography with a Sonos 5500 or 7500 ultrasound system (Philips, Andover, MA, USA). All echocardiography studies were performed by an experienced echocardiographer who was blinded to the results of the plasma BNP measurement. 2D echocardiography was done to assess the left ventricular wall motion from different views. If regional wall motion abnormalities of the left ventricle existed, the left ventricular EF was measured by biplane Simpson's rule. Possible causes of ventricular dysfunction such as anomalous origin of the left coronary artery from the pulmonary artery and severe ventricular outflow obstruction were excluded.

2.4. Plasma BNP measurement

A rapid, commercially available BNP fluorescence immunoassay (Triage Assay, Biosite Diagnostics, Inc., San Diego, CA, USA) approved by the US Food and Drug Administration was used for the BNP measurements in this study. For this point-of-care assay, a venous blood sample was collected into an ethylenediaminetetraacetic acid (EDTA) tube and was analyzed at room temperature within 30 min of collection. BNP levels between 5 pg/ml and 5000 pg/ml can be measured using this kit, with an average inter-assay coefficient of variation of 3%. BNP standard deviation scores (SDS) or *z*-scores were calculated according to the following formulas: boys and girls aged 2 weeks to 10 years: BNP SDS = (natural logarithm (ln) of BNP plasma level − 2.05)/0.56; female patients older than 10 years: BNP SDS = (lnBNP − 2.32)/0.67; male patients older than 10 years: BNP SDS = (lnBNP − 1.80)/0.31.[20] In this study, blood was drawn from a peripheral vein or arterial line within 24 h after being admitted to the PICU for patients with a severe EV71 infection diagnosed clinically and confirmed by laboratory methods later, for the determination of the plasma BNP level; this was repeated if the disease progressed. In the control group, blood was drawn from a peripheral vein soon after informed consent was obtained.

2.5. Statistical analysis

Categorical variables were summarized as the median, mean ± standard deviation, or case numbers. Statistical analyses were performed with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparisons of demographic data, laboratory features, and echocardiography results between the study groups were analyzed by Kruskal–Wallis test, one-way analysis of variance (ANOVA) with post-hoc multiple comparison Scheffé test, or Mann–Whitney *U*-test for continuous variables, and Pearson's Chi-square test or the Chi-square test for trends. Bars with different letters indicated significant differences between groups. Because of the different ages and sizes of patients, the echocardiography parameters of left ventricular end-diastolic and end-systolic internal dimensions (LVEDd and LVEDs) were adjusted for body surface area for comparisons.[22] Cut-off points for receiver operating characteristic curve analyses, expressing sensitivity, specificity, the positive predictive value (PPV), and the negative predictive value (NPV), were determined. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 53 children (33 males and 20 females) with a mean age of 2.5 ± 1.7 years (range 0.5–6.8 years) were enrolled in this study. The demographic data and laboratory features are shown in Table 1. There were 30 healthy children (median 2.2 years) in group 1, 20 children (median 1.6 years) with EV71 stage 2 infections in group 2, and three children (median 3.0 years) with EV71 stage 3 infections in group 3. There were no statistically significant differences in gender, age, body length, body weight, and body surface area among the groups. There were no significant differences in cardiothoracic ratio calculated from the chest radiography, creatine kinase value, or C-reactive protein level between group 2 and group 3, but patients in group 3 had higher heart rate ($p = 0.006$), systolic blood pressure ($p = 0.028$), MB fraction of creatine kinase (CK-MB) ($p = 0.025$), and troponin I ($p = 0.001$) values than those in group 2. All patients in

Table 1
Demographic and laboratory features of all studied cohorts[a]

	Group 1 (Control, $n=30$)	Group 2 (EV71, stage 2, $n=20$)	Group 3 (EV71, stage 3, $n=3$)	p-Value[b]
Gender	17M/13F	14M/6F	2M/1F	0.632
Age, years	2.5 ± 1.0 (2.2)	2.3 ± 2.4 (1.6)	3.6 ± 2.1 (3.0)	0.056
Body length, cm	89 ± 11 (87)	89 ± 20 (84)	107 ± 19 (97)	0.087
Body weight, kg	13.1 ± 3.2 (12.7)	13.0 ± 6.5 (10.7)	18.0 ± 5.2 (15)	0.064
Body surface area, m^2	0.57 ± 0.10 (0.57)	0.56 ± 0.19 (0.50)	0.73 ± 0.16 (0.64)	0.086
Maximum heart rate, bpm		130 ± 14 (133)	193 ± 15 (195)	0.006
Maximum systolic blood pressure, mmHg		115 ± 10 (111)	136 ± 11 (137)	0.028
Echocardiography				
Left ventricle end-diastolic internal dimension,[c] cm/m^2	5.22 ± 0.57 (5.20)	5.30 ± 0.91 (5.49)	5.39 ± 0.24 (5.42)	0.753
Left ventricle end-systolic internal dimension,[c] cm/m^2	3.11 ± 0.38 (3.16)	3.13 ± 0.63 (3.08)	4.59 ± 0.24 (4.55)	0.015
Ejection fraction (%)	71 ± 5 (70)	73 ± 6 (74)	33 ± 13 (34)	0.007
Fractional shortness (%)	39 ± 4 (38)	41 ± 5 (41)	15 ± 5 (16)	0.007
Cardiothoracic ratio in CXR		0.52 ± 0.03 (0.52)	0.50 ± 0.03 (0.51)	0.382
Laboratory data				
CK (IU/l)		115 ± 81 (68)	262 ± 247 (123)	0.230
CK-MB (IU/l)		13 ± 6 (14)	26 ± 10 (25)	0.025
CRP (mg/dl)		1.66 ± 1.43 (1.22)	1.13 ± 0.84 (1.45)	0.635
Troponin I (ng/ml)		<0.03 (<0.03)	3.84 ± 4.84 (1.48)	0.001
BNP level (pg/ml)	3.8 ± 2.4 (<5)	13.4 ± 12.8 (9.5)	296.3 ± 161.6 (238)	<0.001
BNP z-score	−1.52 ± 0.88 (−2.02)	0.37 ± 1.45 (−0.22)	6.33 ± 0.94 (6.11)	<0.001

BNP, B-type natriuretic peptide; CK, creatine kinase; CK-MB, MB fraction of creatine kinase; CRP, C-reactive protein; CXR, chest X-ray.
[a] Data are presented as mean ± standard deviation (median).
[b] In the p-value column, the comparisons were performed among the groups by Kruskal–Wallis test, one-way analysis of variance (ANOVA) with post-hoc multiple comparison Scheffé test, or Mann–Whitney U-test.
[c] Left ventricle end-diastolic internal dimension and left ventricle end-systolic internal dimension were adjusted for body surface area.

group 2 recovered completely without significant neurological sequelae. No group 3 patients had cardiomegaly on chest radiograms, two patients had pulmonary edema, and all patients had poor left ventricular systolic function with typical regional wall motion abnormalities on echocardiography. Two patients in group 3 were rescued by trans-sternal extracorporeal life support and were successfully weaned off. All of the patients in group 3 survived without cardiac complications, but one had mild weakness of the upper limbs and dysphagia. Among the echocardiography studies, there were no statistically significant differences in adjusted LVEDd among the groups, and no differences in adjusted LVEDs, EF, and FS between group 1 and group 2. The patients in group 3 had significantly larger adjusted LVEDs ($p = 0.015$) and poorer EF ($p = 0.007$) and FS ($p = 0.007$) than those in the other groups, as shown in Figures 1 and 2.

The mean BNP value in group 1 was 3.8 ± 2.4 pg/ml (ranging from <5 pg/ml to 10.4 pg/ml, median <5 pg/ml), in group 2 was 13.4 ± 12.8 pg/ml (ranging from <5 pg/ml to 54.5 pg/ml, median 9.5 pg/ml), and in group 3 was 296.3 ± 161.6 pg/ml (ranging from 172 pg/ml to 479 pg/ml, median 238 pg/mol). The BNP z-scores were −1.52 ± 0.88 (median −2.02), 0.37 ± 1.45 (median −0.22), and 6.33 ± 0.94 (median 6.11), respectively, in the three groups. Results are shown in Table 1. Patients in group 3 had significantly higher plasma BNP levels and BNP z-scores than those in the other groups ($p < 0.001$ and < 0.001, respectively). Although there was no statistically significant difference in the plasma BNP values between group 1 and group 2 ($p = 0.612$), patients in group 2 had significantly higher BNP z-scores than those in group 1 ($p < 0.001$). Among the three groups, the lowest BNP z-score was in group 1 and the highest was in group 3, as shown in Figure 3. Using a BNP value of 100 pg/ml or a BNP z-score of 4.56 to determine the cut-off point for the identification of severe EV71 infection with acute heart failure and EF less than 50%, the sensitivity, specificity, PPV, and NPV were all 100%. Using a BNP z-score of 1.18 (BNP value of 15 pg/ml) to determine the cut-off point for the detection of EV71 stage 2 infection, the sensitivity, specificity, PPV, and NPV were 35%, 100%, 100%, and 70%, respectively.

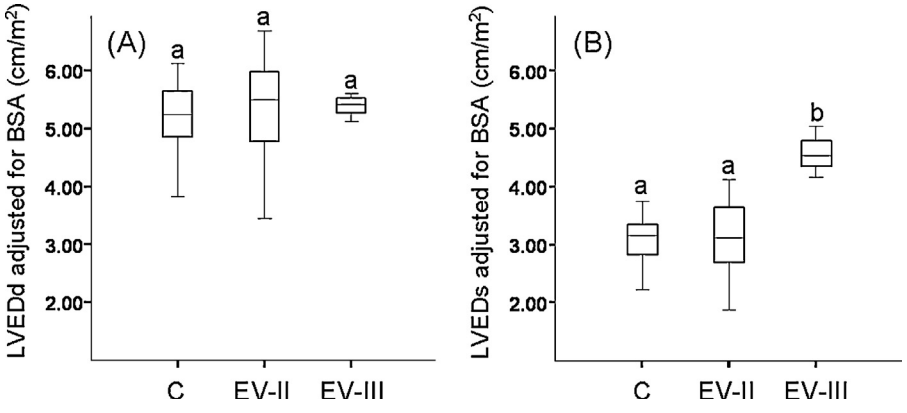

Figure 1. Adjusted left ventricular end-diastolic (A) and end-systolic (B) internal dimensions (LVEDd/LVEDs) categorized by different groups. Differences among groups were analyzed statistically. Bars with the same letters are not significantly different. C, normal controls; EV-II, EV71 stage 2 patients; EV-III, EV71 stage 3 patients.

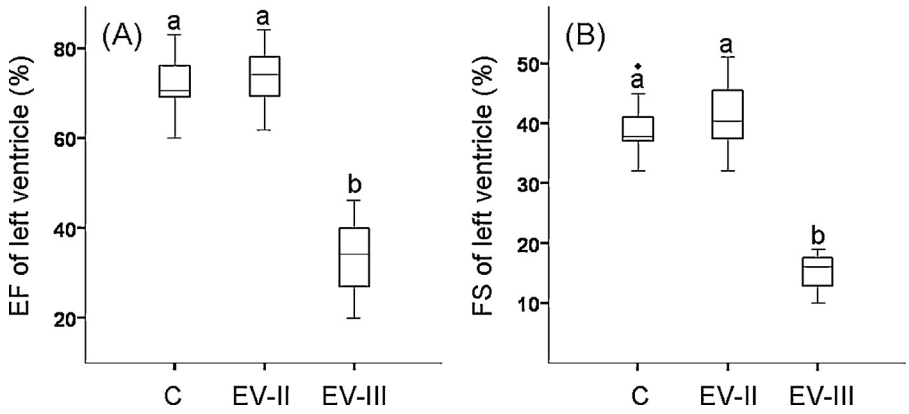

Figure 2. (A) Ejection fraction (EF), and (B) fractional shortness (FS) of the left ventricle categorized by different groups. Differences among groups were analyzed statistically. Bars with the same letters are not significantly different. ◆, Outliers: cases with values that are between 1.5 and 3 box lengths from either end of the box. C, normal controls; EV-II, EV71 stage 2 patients; EV-III, EV71 stage 3 patients.

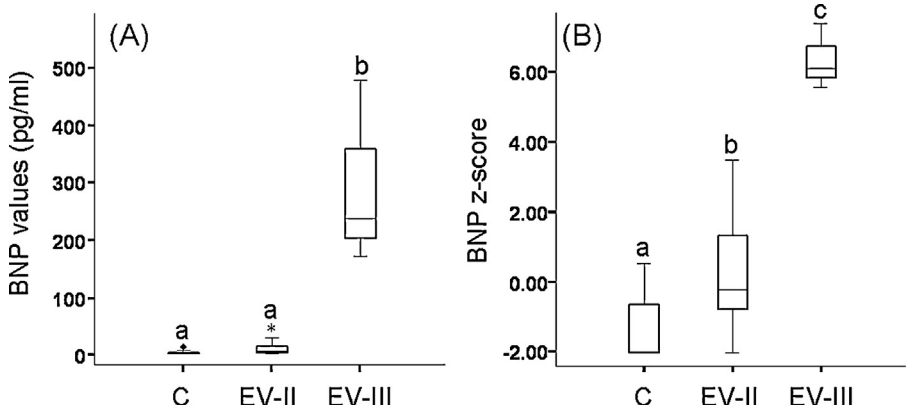

Figure 3. (A) Plasma BNP values, and (B) plasma BNP standard deviation scores categorized by different groups. Differences among groups were analyzed statistically. Bars with the same letters are not significantly different. BNP, B-type natriuretic peptide; ◆, outliers: cases with values that are between 1.5 and 3 box lengths from either end of the box; *, extremes: cases with values more than 3 box lengths from either end of the box. C, normal controls; EV-II, EV71 stage 2 patients; EV-III, EV71 stage 3 patients.

4. Discussion

Children with EV71 infections usually only have oro-mucocutaneous lesions, but a few patients, less than 0.01%, progress to severe EV71 infection. Subsequent acute cardiopulmonary failure has been reported to occur in 11–19% of patients with severe EV71 infections, resulting in a mortality rate of 30–77% and severe neurological sequelae.[1–6,23,24] In this study, all three patients with an EV71 stage 3 infection (group 3) had clinically typical findings, such as normal heart size by chest X-ray and a regional wall motion abnormality of the left ventricle that was identified as 'panic or shivering heart' by echocardiography.[25]

For a biomarker to be valuable in clinical practice, it should be rapidly and accurately measurable at a reasonable cost, add diagnostic or prognostic information to available methods, and help to guide patient management. BNP can fulfill most of these criteria in patients with heart failure and ventricular overload.[26] The Triage test system allows the widespread clinical use of BNP determination, and recent studies have reported the usefulness of BNP assessment in patients with various cardiovascular diseases, including elevated plasma BNP concentrations in adults with heart failure, systolic and diastolic dysfunction, or during different types of hemodynamic overload of the heart. BNP has also been found to be useful for distinguishing cardiac from non-cardiac respiratory distress, to be correlated with the severity of heart failure symptoms, and to predict hospital readmission and mortality.[11–20] However, the clinical application of plasma BNP in children who have severe EV71 infections with or without acute heart failure remains limited.

Although children with severe EV71 infections may experience progression to acute heart failure, symptoms and signs suggestive of heart failure are insensitive and non-specific for diagnosing heart failure.[1–7] The role of BNP testing is clearly defined for diagnosing patients with suspected myocardial stress who have new symptoms of heart failure or acute respiratory distress, and for assessing the prognosis of heart failure patients. In this study, the preliminary results suggest the use of a BNP test to aid in the diagnosis of children who have severe EV71 infections with varying degrees of acute myocardial stress. Comparisons of the echocardiography parameters, including LVEDd, LVEDs, EF, and FS, with plasma BNP values or BNP *z*-scores, showed that there were good correlations of myocardial dysfunction with plasma BNP values and BNP *z*-scores. The plasma BNP values were markedly elevated in patients with EV71 stage 3 infections.

In this study, a BNP value of 100 pg/ml was used as the cut-off point for the detection of severe EV71 with acute heart failure, which is similar to a previously recommended cut-off level for the diagnosis of acute heart failure in adults.[15] However, some limitations of BNP testing have been noted for small elevations of BNP in patients with cor pulmonale, mild ventricular dysfunction, and some congenital heart diseases with left-to-right shunts, as well as in small children.[19,26] Patients with chronic non-cardiac diseases such as severe lung disease, impairment of renal function, and diabetes may have higher BNP concentrations than those of

age-matched controls. Blood concentrations of BNP increase with age, and females have higher BNP values than males in the same age strata. As children grow, BNP levels increase with a positive and powerful linear correlation to age that reflects increasing cardiac load.[27] For the abovementioned reasons, plasma BNP concentrations should be expressed as standard deviation scores calculated according to age- and gender-specific normal values using formulas.[20]

Although there was no significant difference in plasma BNP level between group 1 and group 2, there was a higher BNP *z*-score in group 2 patients who did not actually have a clinical diagnosis of heart failure. We propose that subclinical myocardial dysfunction combined with myocardial stress may have occurred in these patients, resulting in a mild elevation of the plasma BNP level. Compared group 2 patients with group 3 patients and we found that the BNP value was significantly higher in patients with severe EV71 infection and acute heart failure because the pressure loading on their myocardium suddenly increased and the myocardium failed to tolerate the high wall tension and stress. Thus, we used a BNP *z*-score of 1.18 (an approximate BNP value of 15 pg/ml) as the cut-off point for the identification of EV71 stage 2 infection. The sensitivity and specificity were 35% and 100%, respectively, which suggests that a lower value of BNP may not be accurate enough to rule out an EV71 stage 2 infection. Although the BNP *z*-score can be derived from formulas, the BNP *z*-score would not be suitable in routine clinical practice because it requires a more complex calculation, so we suggest using a BNP value >15 pg/ml as a predictor of EV71 stage 2 infection.

BNP is not a stand-alone test; it is of greatest value when it complements clinical judgment along with other available tests, such as cardiac troponins, CK-MB, and echocardiography. The use of the BNP test in conjunction with other clinical information may lead to more accurate and early diagnosis of severe EV71 infections with acute myocardial stress.

Some limitations of this study need to be considered. In this study mildly elevated plasma BNP values were found in EV71 stage 2 patients who did not actually have a clinical diagnosis of heart failure. A control group of children with uncomplicated EV71 infections would have helped to clarify this situation. However, we do not routinely isolate the virus and identify enteroviruses for uncomplicated HFMD/herpangina patients. We will consider enrolling uncomplicated EV71 infection patients as a control group in a further study. Although we found plasma BNP values to be significantly elevated in children with severe EV71 infections with acute heart failure, the number of children in group 3 was too small to determine reference values. Further investigations of plasma BNP levels detected at the different stages of EV71 infection are needed to understand the trends in plasma BNP levels with regard to disease progression and convalescence; large multi-institutional studies are required.

In conclusion, our findings validate and extend the observations made in a leading study using a BNP test, a powerful cardiac biomarker, to aid in the diagnosis of children who have severe EV71 infections with acute heart failure. The results of this study suggest that rapid measurement of the BNP level in blood can demonstrate the relationship of BNP levels with the severity and speed of heart failure, and can improve the ability of clinicians to establish whether or not children with severe EV71 infections have acute heart failure. We speculate that a plasma BNP value >100 pg/ml could identify children with severe EV71 infections who have marked left ventricular dysfunction and who need further evaluation as well as early interventions, such as extracorporeal life support. In order to reach definitive conclusions regarding the clinical utility of BNP as a biomarker of heart involvement in children with severe EV71 infections, further investigations and large multi-institutional studies are necessary.

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Ethical approval: This study was approved by the Institutional Review Board of the Taichung Veterans General Hospital.

Conflict of interest: The authors hereby declare that no conflict of interest exists.

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